SUBCLINICAL INFECTION OF DOGS BY CANINE-ADAPTED MEASLES VIRUS EVIDENCED BY THEIR SUBSEQUENT IMMUNITY TO CANINE DISTEMPER VIRUS

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ABSTRACT

MOURA, ROBERTO A. (Chas. Pfizer and Company, Inc., Terre Haute, Ind.) AND JOEL WARREN. Subclinical infection of dogs by canine-adapted measles virus evidenced by their subsequent immunity to canine distemper virus. J. Bacteriol. **82**:702–705. 1961.—Young dogs were inoculated with virulent measles virus which had been adapted to canine kidney or human amnion cell culture. None of the animals showed any clinical symptoms nor could virus be isolated from the blood, although measles-neutralizing and complement-fixing antibodies developed during convalescence. All dogs failed to develop antibody to canine distemper. However, when these and normal control animals were subsequently inoculated intracerebrally with virulent distemper virus, each of the controls succumbed to typical symptoms, whereas all of the measles-immune dogs survived. These results suggest that the cross-protection conferred by measles against canine distemper virus infection involves factors other than humoral antibody. The immunity persists for a considerable length of time.

The possible role of canines in the dissemination of measles in man is a natural question, following the demonstration of infection in dogs by measles virus, as manifested by the development of circulating antibody and resistance to the antigenically related virus of canine distemper (Warren et al., 1960; Gillespie and Karzon, 1960). The problem might be clarified if the dog could be shown to experience a phase of viremia during infection or to develop overt signs of disease. Ryazantseva (1956) has reported a mild febrile illness in puppies, with rash and viremia, when the inoculum was blood or throat washings from cases of measles. In contrast, others have not observed clinically recognizable disease when measles virus grown in monkey kidney tissue culture was inoculated subcutaneously or intraperitoneally (Warren et al., 1960), or intravenously (Gillespie and Karzon, 1960). The presence of circulating virus was not sought for in these studies.

In the present investigation we have attempted unsuccessfully to induce a symptomatic illness in young dogs by the injection of a strain of measles virus serially passaged in canine kidney and human amnion cell cultures. The former cell was used because it was believed that some degree of adaptation to canine tissue might occur. Although measles antibody developed, and the animals were subsequently immune to canine distemper challenge as reported earlier, none of these dogs showed any symptoms referable to measles infection.

MATERIALS AND METHODS

Viruses. Two lines of measles virus were used, both derived from the Edmonston strain (Enders and Peebles, 1954). One line had been passaged 24 times in human kidney and 40 times in human amnion primary cell cultures. The other had received an additional nine passages in dog kidney tissue culture. The viruses had titers of $10^{-5.0}$ and $10^{-4.5}$ $TCID_{50}$ per ml, respectively, when titrated on a serial passage human amnion cell line (AV3). The identity of both lines was established by means of neutralization with human convalescent measles serum and monkey hyperimmune measles serum.

The Snyder Hill strain of canine distemper virus (Gillespie and Rickard, 1956) was obtained through the kindness of A. L. Brueckner. It was maintained in dogs by serial intracerebral transfers of a 10% suspension of canine brain and spleen. After inoculation of 0.5 ml intracerebrally, this virus consistently produced either clinical symptoms or death in 10 to 20 days.

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Twenty-three mongrel puppies, 8 to 10 weeks of age, were divided into three groups as follows: Group A (11 dogs) was inoculated with the human line of measles virus; group B (10 dogs) was inoculated with the dog-kidney tissue culture line; group C (2 dogs) was not inoculated and served as normal controls.

Each group was further subdivided on the basis of the route of inoculation. In group A there were subgroups: (a) consisting of three dogs which were inoculated intracerebrally with 0.1 ml of virus under general anesthesia; (b) three dogs, inoculated intravenously with 1.5 ml of virus; (c) three dogs, inoculated with 2.0 ml of virus subcutaneously; and (d) dogs inoculated by all three routes combined.

In group B there were also four subgroups representing the different routes of inoculation. Here the numbers of animals per subgroup were: (a) one; (b) three; (c) three; and (d) three. The normal dogs (group C) were kept in the same quarters with the inoculated ones. All animals were carefully observed, and rectal temperatures were taken on days 0, 3, 5, 7, 14, 21, and 28.

The dogs were bled on days 0, 7, 14, 21, and 28, and the sera were stored at −20 C. In those animals that received combined inoculations (subgroup d), blood samples were collected for virus isolation and for differential white blood cell counts on days 0, 3, 4, 5, 6, and 7.

Specimens for virus isolations were stored at −70 C until studied. A human amnion serial cell line (AV3) was used for attempted detection of viremia. These were inoculated with 0.2 ml of heparinized blood. Blind passages were made from these after 1 week, and both the primary and secondary cultures were observed for an additional 2 weeks.

Distemper challenge. Seventy-seven days after measles infection, animals of groups A, B, and C were bled and intracerebrally injected with 0.5 ml of a 10% suspension of canine distemper virus. At that time, three new, nonvaccinated mongrel puppies were included for virus controls. On the 98th day all surviving dogs were bled.

Serology. In all instances the serum samples from one dog were tested simultaneously. Measles virus neutralization tests were performed in human amnion serial cell cultures, using as the challenge virus 1,000 to 10,000 $TCID_{50}$ of a HeLa cell-adapted line of measles. Equal volumes of virus suspension and serum dilutions were mixed and held for 1 hr and 4 C; 0.2 ml of the mixture was then inoculated into each of three culture tubes. The neutralization end point was read at that time when the control titration of the challenge virus showed cytopathic changes at the dilutions between 10 to 100 $TCID_{50}$ per ml (Black, 1959).

Measles complement fixation tests were carried out by the Fulton-Dumbell contour method on plastic plates (Fulton, 1958). Approximately two to four units of antigen, consisting of fluid from measles-infected stable human amnion cultures (AV3), were employed.

RESULTS

Careful clinical examination of dogs inoculated with measles virus by any route failed to reveal symptoms of disease. Virus was never isolated from the blood. The temperature of all animals remained within the normal range, and total and differential leucocyte counts showed no deviation from a normal pattern. Neutralizing and complement-fixing antibodies began to appear about the 14th day after infection, and by the 77th day the sera of all inoculated dogs had neutralizing titers ranging from 1:10 to 1:800. Although the number of animals per group was minimal, differences in titer were not associated with the strain of virus or the route of its injection. At this time the animals were challenged with live distemper virus. During the following 2 weeks there was no further increase of neutralizing or complement-fixing titers against measles virus (Table 1).

Through the kind assistance of David Karzon, neutralization tests for canine distemper virus (CDV) were performed on all of the dog sera taken before, and from 1 to 4 weeks after, measles infection. All animals failed to develop CDV antibody during this interval.

Upon challenge with CDV, each of five control dogs succumbed with classic symptoms of this disease within 18 to 22 days, whereas all of the dogs exposed to measles virus remained well. It was of considerable interest that animals with even the lowest levels of measles-neutralizing antibody (no. 13, 17, and 20) were immune to the severe challenge of direct injection of virulent CDV into the central nervous system, an observation which agrees with earlier findings (Warren et al., 1960; Gillespie and Karzon, 1960).

TABLE 1. *Development of antibodies to measles virus in dogs infected by various routes*

Source of virus	Route	Dog	Day													
			0		7		14		21		28		77*		98	
			N	CF	N	CF	N	CF	N	CF	N	CF	N	CF	N	CF
Human amnion cell culture	Intracerebral	1	0	0	0	0	10	50	10	48	10	32	200	18	200	10
		2	0	0	0	0	0	0	10	0	10	0	100	0	100	0
		3	0	0	0	0	10	0	100	16	100	32	400	40	400	—
	Intravenous	4	0	0	0	0	10	0	10	32	10	32	100	28	100	15
		5	0	0	0	0	50	20	50	38	50	40	100	50	100	150
		6	0	0	0	0	10	8	100	64	200	128	800	120	800	50
	Subcutaneous	7	0	0	0	0	10	0	10	0	10	0	—	—	—	—
		8	0	0	0	0	0	0	10	19	10	32	—	—	—	—
		9	0	0	0	0	0	0	10	0	10	0	200	0	200	0
	Combined	11	0	0	0	0	10	0	10	0	10	0	100	0	100	0
		12	0	0	0	0	50	86	50	115	100	120	800	128	800	90
Canine kidney cell culture	Intracerebral	13	0	0	0	0	10	0	10	0	10	19	50	8	50	8
	Intravenous	16	0	0	0	0	10	0	10	25	50	25	0	0	—	—
		17	0	0	0	0	0	0	10	0	10	0	10	0	10	0
		18	0	0	0	0	10	0	10	30	10	70	200	92	—	—
	Subcutaneous	19	0	0	0	0	10	13	10	15	10	18	100	80	100	100
		20	0	0	0	0	0	0	0	0	10	0	50	0	50	0
		21	0	0	0	0	0	16	10	28	10	128	—	—	—	—
	Combined	22	0	0	0	0	10	0	10	15	10	50	800	100	800	128
		23	0	0	0	0	10	0	50	0	50	19	400	280	—	—
		24	0	0	0	0	0	0	10	0	10	0	100	0	100	0
None		25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		26	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Distemper challenge. N = 0 = Neutralization titer < 1:10; CF = 0 = Complement fixation titer < 1:8.

DISCUSSION

The complete absence of clinical signs in dogs infected with a line of measles virus that had been serially passaged in canine tissue culture was not expected. We believe that because of technical factors or searching in the wrong site we may have failed to detect some persistence or multiplication of measles virus in these animals. In particular, we intend to inspect the inoculation site, the kidneys, and the lymphatic system in future work. There was no apparent cross-infection among the dogs, since the two normal controls failed to develop measles antibodies and were killed by challenge with the Snyder-Hill virus.

A puzzling finding, previously noted by Gillespie and Karzon (1960), was the presence of a high level of immunity to distemper virus in the absence of antibody to this virus. These authors have suggested interference between CDV and measles as a possible explanatory mechanism. This interesting immunological phenomenon merits additional study, since this type of local cellular resistance was maintained for as long as 3 months.

The possible value of measles vaccine, live or inactivated, in the prophylaxis of canine distemper is again suggested by these findings. The well-known variability in potency and effectiveness of commercially available attenuated canine distemper vaccine (Larin, 1955; Oxer, 1959) accentuates the need for improvement in current methods of distemper prevention.

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